Molecular cloning of human cardiac troponin I using polymerase chain reaction.

Vallins WJ, Brand NJ, Dabhade N, Butler-Browne G, Yacoub MH, Barton PJ.

Department of Cardiothoracic Surgery, National Heart and Lung Institute, London, UK.

We have used the polymerase chain reaction (PCR) to synthesise a cDNA encoding part of human cardiac troponin I. Amplification was achieved using fully degenerate sets of oligonucleotides corresponding to conserved regions of amino acid sequence identified in other troponin I isoforms. The cloned PCR fragment was subsequently used to isolate full-length cDNAs from a cardiac cDNA library. We describe the approach, as a general cloning strategy starting from limited amino-acid sequence data and report the cloning, and complete amino acid sequence of human cardiac troponin I. Analysis of human development using these clones demonstrates early expression of this gene in the heart.

PMID: 2226790 [PubMed - indexed for MEDLINE]

NMR analysis of cardiac troponin C-troponin I complexes: effects of phosphorylation.

Finley N, Abbott MB, Abusamhadneh E, Gaponenko V, Dong W, Gasmi-Seabrook G, Howarth JW, Rance M, Solaro RJ, Cheung HC, Rosevear PR.

Department of Molecular Genetics, Biochemistry, and Microbiology, University of Cincinnati, College of Medicine, OH 45267, USA.

Phosphorylation of the cardiac specific amino-terminus of troponin I has been demonstrated to reduce the Ca2+ affinity of the cardiac troponin C regulatory site. Recombinant N-terminal cardiac troponin I proteins, cardiac troponin I(33-80), cardiac troponin I(1-80), cardiac troponin I(1-80)DD and cardiac troponin I(1-80) pp, phosphorylated by protein kinase A, were used to form stable binary complexes with recombinant cardiac troponin C. Cardiac troponin I(1-80)DD, having phosphorylated Ser residues mutated to Asp, provided a stable mimetic of the phosphorylated state. In all complexes, the N-terminal domain of cardiac troponin I primarily makes contact with the C-terminal domain of cardiac troponin C. The nonphosphorylated cardiac specific amino-terminus, cardiac troponin I(1-80), was found to make additional interactions with the N-terminal domain of cardiac troponin C.

PMID: 10403385 [PubMed - indexed for MEDLINE]

1: Anal Biochem 2002 Jul 1;306(1):92-9 Related Articles, Books LinkOut

FULL-TEXT ARTICLE

Separation of synthetic oligonucleotide dithioates from monothiophosphate impurities by anion-exchange chromatography on a mono-q column.

Yang X, Hodge RP, Luxon BA, Shope R, Gorenstein DG.

Sealy Center for Structural Biology

A method using a strong anion-exchange liquid-chromatography column, Mono-Q, has been developed for high-resolution analysis and purification of oligonucleotide dithioates, which were synthesized by an automated, solid-phase, phosphorothioamidite chemistry. High-resolution separation of oligonucleotide phosphorodithioates from monothiophosphate impurities was obtained. High-resolution separation was also demonstrated at pH 8. The separation of oligonucleotide dithioates was found to be linearly dependent on the number of sulfurs for the same sequence length. Thiocyanate, SCN(-), as eluting anion, can be used to purify oligonucleotides containing a high percentage of phosphorodithioate linkages in lower salt concentrations and provides better separation than chloride as eluting anion. (c) 2002 Elsevier Science (USA).

PMID: 12069419 [PubMed - in process]

Display Abstract Sort Save Text Clip Add Order

Modified aluminas as chromatographic supports for high-performance liquid chromatography.

Pesek JJ, Matyska MT.

Department of Chemistry, San Jose State University, College of Science, CA 95192-0101, USA.

This review begins by describing the relevant properties of alumina as a support material for chemically bonded stationary phases in HPLC. The most common chemical modification processes are summarized as well as the advantages and disadvantages of each method. In order to more fully understand the chemically modified alumina surface, some spectroscopic approaches are outlined for characterization of the bonded phases. Finally, a number of successful applications are described for a variety of chemically modified aluminas in order to illustrate their potential usefulness and to compare their chromatographic behavior to the more conventional silica-based materials.

PMID: 12064521 [PubMed - in process]

Entrez PubMed
Overview
Help | FAQ
Tutorial
New/Noteworthy
E-Utilities

PubMed Services
Journal Browser
MeSH Browser
Single Citation Matcher
Batch Citation Matcher
Clinical Queries
LinkOut
Cubby

Related Resources
Order Documents
NLM Gateway
TOXNET
Consumer Health
Clinical Alerts
ClinicalTrials.gov
PubMed Central

Privacy Policy

1: Fitzsimons DP, Patel JR, Campbell KS, Moss RL. Related Articles

Cooperative mechanisms in the activation dependence of the rate of force development in rabbit skinned skeletal muscle fibers.
J Gen Physiol. 2001 Feb;117(2):133-48.
PMID: 11158166 [PubMed - indexed for MEDLINE]

2: Schriemer DC, Yalcin T, Li L. Related Articles

MALDI mass spectrometry combined with avidin-biotin chemistry for analysis of protein modifications.
Anal Chem. 1998 Apr 15;70(8):1569-75.
PMID: 9569766 [PubMed - indexed for MEDLINE]

3: Fuchs F, Liou YM, Grabarek Z. Related Articles

The reactivity of sulfhydryl groups of bovine cardiac troponin C.
J Biol Chem. 1989 Dec 5;264(34):20344-9.
PMID: 2584219 [PubMed - indexed for MEDLINE]

4: Leszyk J, Collins JH, Leavis PC, Tao T. Related Articles

Cross-linking of rabbit skeletal muscle troponin with the photoactive reagent 4-maleimidobenzophenone: identification of residues in troponin I that are close to cysteine-98 of troponin C.
Biochemistry. 1987 Nov 3;26(22):7042-7.
PMID: 3427058 [PubMed - indexed for MEDLINE]

5: Byers DM, Kay CM. Related Articles

Hydrodynamic properties of bovine cardiac troponin-I and troponin-T.
J Biol Chem. 1983 Mar 10;258(5):2951-4.
PMID: 6826548 [PubMed - indexed for MEDLINE]

6: Murakami U, Uchida K, Hiratsuka T. Related Articles

Cardiac myosin from pig heart ventricle. Purification and enzymatic properties.
J Biochem (Tokyo). 1976 Sep;80(3):611-9.
PMID: 10292 [PubMed - indexed for MEDLINE]

7: Parker CJ Jr, Kilbert LH Jr. Related Articles

A study of the role of sulfhydryl groups in the interaction of troponin and myofibrils.
Arch Biochem Biophys. 1970 Oct;140(2):326-33. No abstract available.
PMID: 4248628 [PubMed - indexed for MEDLINE]

8: Ebashi S, Kodama A, Ebashi F. Related Articles

Troponin. I. Preparation and physiological function.
J Biochem (Tokyo). 1968 Oct;64(4):465-77. No abstract available.
PMID: 5707834 [PubMed - indexed for MEDLINE]

1: Proteomics 2002 Jan;2(1):22-31

Related Articles, Books, LinkOut

Application of reversed phase high performance liquid chromatography for subproteomic analysis of cardiac muscle.

Neverova I, Van Eyk JE.

Department of Physiology, Queen's University, Kingston, ON, Canada.

The application of protein separation methodologies, such as reversed phase chromatography, should allow differential separation of the proteome, or at least specific subproteomes, comparable to that achieved by two-dimensional electrophoresis (2-DE). A rapid sequential protein extraction method (termed "IN Sequence") was developed to isolate three distinct subproteomes of cardiac muscle. Two subproteomes, those enriched for the cytoplasmic or myofilament proteins, can be separated by either reversed phase high performance liquid chromatography (RP-HPLC) or 2-DE. Reversed phase HPLC of the myofilament protein enriched extract was optimized for resolution and peak numbers by altering flow rate, gradient rate and the organic modifiers, isopropanol and acetronitrile. The myofilament protein enriched extract from failing swine heart, due to coronary artery ligation (LAD), was compared to the extract from a sham operated animal (SHAM). The HPLC chromatograms of these extracts were similar, but distinctive in many regions. The HPLC fractions, collected within some of these distinct regions of the chromatograms were analyzed using peptide mass fingerprinting - mass spectrometry and immunoblot analysis. Two myofilament proteins, troponin T and myosin heavy chain, were identified and found differentially modified in the SHAM and LAD hearts. Both troponin T and myosin heavy chain are problematic proteins for 2-DE, but yet they were resolved by reversed phase chromatography. Therefore, RP-HPLC can be used in conjunction with 2-DE to enhance protein separation of myofilament protein subproteome.

PMID: 11788988 [PubMed - indexed for MEDLINE]

Display Abstract Sort Save Text Clip Add Order

Overexpression of human cardiac troponin-I and troponin-C in Escherichia coli and their purification and characterisation. Two point mutations allow high-level expression of troponin-I.

al-Hillawi E, Minchin SD, Trayer IP.

School of Biochemistry, University of Birmingham, England.

We have overexpressed human cardiac troponin-I in Escherichia coli. Initially, protein expression was not detected in the bacterial cell extracts. Systematic deletion of the N-terminal region of the protein generated a series of truncated mutants which were expressed at varying levels in the bacteria. This allowed us to narrow the problem down to the first five codons in the gene sequence. In order to achieve expression at high levels, two base changes were required, in the second and the fourth codons of the cDNA sequence. The codon changes, (Ala2) GCG-->GCC and (Gly4) GGG-->GGT, do not alter the coding potential of the DNA. We have also overexpressed the human cardiac isoform of troponin-C. Both proteins were purified using ion-exchange chromatography and have been proved to be biologically active. The recombinant troponin-I was able to bind to a troponin-C affinity column in the presence of 9 M urea in a calcium-dependent manner. The calcium-dependent troponin-I-troponin-C complex between both recombinant proteins was also demonstrated by alkaline-urea gel electrophoresis. In addition, troponin-I inhibited the acto-S1 Mg-ATPase activity; this inhibition was potentiated by the presence of tropomyosin and was reversed by the addition of troponin-C to the system. Biological activity was also demonstrated in vivo in that the recombinant proteins were able to restore the calcium-dependent force generation to calcium-insensitive skinned muscle fibres.

PMID: 7957210 [PubMed - indexed for MEDLINE]

1: al-Hillawi L, Minchin SD, Trayer IP. Related Articles

Overexpression of human cardiac troponin-I and troponin-C in Escherichia coli and their purification and characterisation. Two point mutations allow high-level expression of troponin-I.
Eur J Biochem. 1994 Nov 1;225(3):1195-201.
PMID: 7957210 [PubMed - indexed for MEDLINE]

2: Quaggio RB, Ferro JA, Monteiro PB, Reinach FC. Related Articles, Nucleotide, Protein

Cloning and expression of chicken skeletal muscle troponin I in Escherichia coli: the role of rare codons on the expression level.
Protein Sci. 1993 Jun;2(6):1053-6. No abstract available.
PMID: 8318890 [PubMed - indexed for MEDLINE]

3: Armour KL, Harris WJ, Tempest PR. Related Articles, Nucleotide, Protein

Cloning and expression in Escherichia coli of the cDNA encoding human cardiac troponin I.
Gene. 1993 Sep 15;131(2):287-92.
PMID: 8406024 [PubMed - indexed for MEDLINE]

4: Liu S, Zhang MY, Song Q, Zhang X, Kadijevic L, Shi Q. Related Articles

Extra leader sequence affects immunoactivity of cardiac troponin I.
Clin Chem. 1999 Aug;45(8 Pt 1):1300-2. No abstract available.
PMID: 10430804 [PubMed - indexed for MEDLINE]

5: Malnic B, Reinach FC. Related Articles

Assembly of functional skeletal muscle troponin complex in Escherichia coli.
Eur J Biochem. 1994 May 15;222(1):49-54.
PMID: 8200352 [PubMed - indexed for MEDLINE]

6: Reiffert S, Maytum R, Geeves M, Lohmann K, Greis T, Bluggel M, Meyer HE, Heilmeyer LM, Jaquet K. Related Articles

Characterization of the cardiac holotroponin complex reconstituted from native cardiac troponin T and recombinant I and C.
Eur J Biochem. 1999 Apr;261(1):40-7.
PMID: 10103031 [PubMed - indexed for MEDLINE]

7: Zhang R, Zhao J, Potter JD. Related Articles

Phosphorylation of both serine residues in cardiac troponin I is required to decrease the Ca2+ affinity of cardiac troponin C.
J Biol Chem. 1995 Dec 22;270(51):30773-80.
PMID: 8530519 [PubMed - indexed for MEDLINE]

8: Xu GQ, Hitchcock-DeGregori SE. Related Articles

Synthesis of a troponin C cDNA and expression of wild-type and mutant proteins in Escherichia coli.
J Biol Chem. 1988 Sep 25;263(27):13962-9.

PMID: 2971047 [PubMed - indexed for MEDLINE]

9: Fujita-Becker S, Kluwe L, Miegel A, Maeda K, Maeda Y. Related Articles

Reconstitution of rabbit skeletal muscle troponin from the recombinant subunits all expressed in and purified from E. coli.
J Biochem (Tokyo). 1993 Sep;114(3):438-44.
PMID: 8282738 [PubMed - indexed for MEDLINE]

10: Hayden M, Traphagen L, Wilkins J, Schmitz E, Laird D, Herrmann R, Mandecki W. Related Articles

Expression in Escherichia coli and affinity purification of a CKS-troponin I fusion protein.
Protein Expr Purif. 1995 Jun;6(3):256-64.
PMID: 7663159 [PubMed - indexed for MEDLINE]

11: Wilkinson JM. Related Articles, Protein

Troponin C from rabbit slow skeletal and cardiac muscle is the product of a single gene.
Eur J Biochem. 1980 Jan;103(1):179-88.
PMID: 7358047 [PubMed - indexed for MEDLINE]

12: Ramakrishnan S, Hitchcock-DeGregori SE. Related Articles

Investigation of the structural requirements of the troponin C central helix for function.
Biochemistry. 1995 Dec 26;34(51):16789-96.
PMID: 8527454 [PubMed - indexed for MEDLINE]

13: Malnic B, Farah CS, Reinach FC. Related Articles

Regulatory properties of the NH2- and COOH-terminal domains of troponin T. ATPase activation and binding to troponin I and troponin C.
J Biol Chem. 1998 Apr 24;273(17):10594-601.
PMID: 9553120 [PubMed - indexed for MEDLINE]

14: Straceski AJ, Nakouzi AS, Malhotra A. Related Articles

Expression of regulated cardiac troponin I in Escherichia coli.
J Mol Cell Cardiol. 1994 Dec;26(12):1565-72.
PMID: 7731051 [PubMed - indexed for MEDLINE]

15: Martin AF, Orlowski J. Related Articles, Nucleotide, Protein

Molecular cloning and developmental expression of the rat cardiac-specific isoform of troponin I.
J Mol Cell Cardiol. 1991 May;23(5):583-8.
PMID: 1886137 [PubMed - indexed for MEDLINE]

16: Toyota N, Shimada Y, Bader D. Related Articles, Nucleotide, Protein

Molecular cloning and expression of chicken cardiac troponin C.
Circ Res. 1989 Nov;65(5):1241-6.
PMID: 2805242 [PubMed - indexed for MEDLINE]

17: Finley N, Abbott MB, Abusamhadneh E, Gaponenko V, Dong W, Gasmi-Seabrook G, Howarth JW, Rance M, Solaro RJ, Cheung HC, Rosevear PR. Related Articles

NMR analysis of cardiac troponin C-troponin I complexes: effects of phosphorylation.
FEBS Lett. 1999 Jun 18;453(1-2):107-12.
PMID: 10403385 [PubMed - indexed for MEDLINE]

18: Guo X, Wattanapermpool J, Palmiter KA, Murphy AM, Solaro RJ. Related Articles, Nucleotide, Protein

Mutagenesis of cardiac troponin I. Role of the unique NH2-terminal peptide in myofilament activation.

J Biol Chem. 1994 May 27;269(21):15210-6.
PMID: 8195157 [PubMed - indexed for MEDLINE]

19: Katayama E, Nozaki S. Related Articles

Ca2+-dependent binding of synthetic peptides corresponding to some regions of troponin-I to troponin-C.
J Biochem (Tokyo). 1982 Apr;91(4):1449-52.
PMID: 7096299 [PubMed - indexed for MEDLINE]

20: Vallins WJ, Brand NJ, Dabhade N, Butler-Browne G, Yacoub MH, Barton PJ. Related Articles, Nucleotide, OMIM, Protein

Molecular cloning of human cardiac troponin I using polymerase chain reaction.
FEBS Lett. 1990 Sep 17;270(1-2):57-61.
PMID: 2226790 [PubMed - indexed for MEDLINE]

Entrez PubMed
Overview
Help : FAQ
Tutorial
New/Noteworthy
E-Utilities

PubMed Services
Journal Browser
MeSH Browser
Single Citation Matcher
Batch Citation Matcher
Clinical Queries
LinkOut
Cubby

Related Resources
Order Documents
NLM Gateway
TOXNET
Consumer Health
Clinical Alerts
ClinicalTrials.gov
PubMed Central

Privacy Policy

Reconstitution of rabbit skeletal muscle troponin from the recombinant subunits all expressed in and purified from E. coli.

Fujita-Becker S, Kluwe L, Miegel A, Maeda K, Maeda Y.

European Molecular Biology Laboratory, Desy, Hamburg, Germany.

Three subunits of rabbit skeletal muscle troponin were expressed in and purified from Escherichia coli. The procedures were optimized, and the reconstituted troponin complex is highly homogeneous, stable, and obtainable in large quantities, allowing us to conduct crystallization studies of the troponin complex. The three subunits expressed and purified are beta-TnT(N'-208), TnI(C64A, C133S), and the wild type TnC. beta-TnT(N'-208) is a 25 kDa fragment of beta-troponin T, which consists of 208 amino acids and lacks 58 residues in the N-terminal variable region. TnI(C64A, C133S) is a mutant troponin I, in which Cys-64 and Cys-133 are replaced by Ala and Ser, respectively. Each subunit was separately expressed in E. coli, purified by column chromatography including HPLC, and reassembled to form troponin complex. The reconstituted troponin complex was not distinguishable from authentic troponin prepared from rabbit skeletal muscle: the acto-S1 ATPase rate, as well as the superprecipitation, was calcium-sensitive. Small flat crystals up to 0.2 mm long have been reproducibly obtained in preliminary crystallization trials.

PMID: 8282738 [PubMed - indexed for MEDLINE]